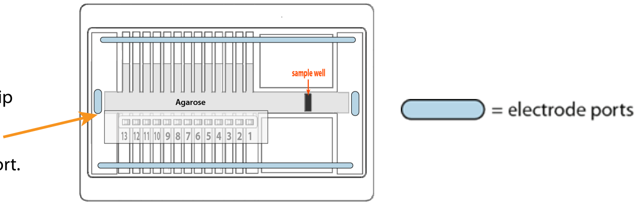
8. From left-to-right, seal the elution modules with the adhesive tape provided. Firmly rub the tape with a smooth round plastic object (like a lab marker pen) to firmly seal the elution elution wells.

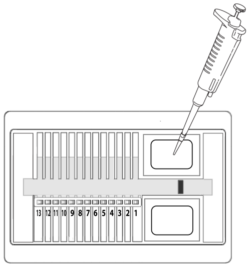


Make sure adhesive strip does not obstruct electrode port.

9. Carefully move the cassette to the SageELF nest.

10. Top up the liquid level in the upper buffer area. Add buffer until it is completely full (>2ml). This is a critical step, refer the operation manual if unfamiliar with the proper level.

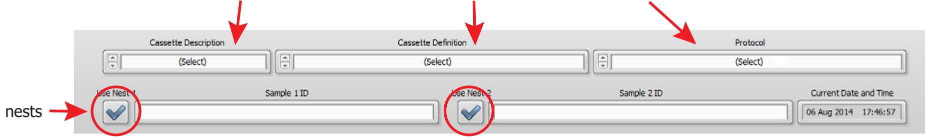
11. Remove 1.0 ml of buffer from the upper buffer area to set the correct volume.



Top off buffer in the cassette by pippetting in to the upper buffer area.

E. Run the Electorphoresis Current Test

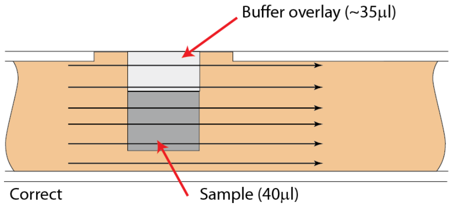
1. Close the lid.
2. In the Main Tab, press "Clear Run Data".
3. Select: the Cassette Description, Cassette Definition, Protocol, and Nests to be run.



4. Press "Check Current". When the test is complete, open the lid.

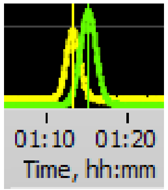
F. Load Sample(s)

1. Make sure the sample well is full. If not, top it off with buffer. Remove 40µl of buffer from the sample well, and load 40µl of sample into that well. Make sure the sample well is full after loading.



G. Run

1. Close the lid.
2. Press "Run Protocol".
3. During a run, a marker peak should be detected for each cassette that is run. The marker runs ahead of the fractionation range.



Marker 03

SageELF™ Quick Guide

3% SDS-Agarose Gel Cassette

for protein fractionation between 10kDa - 300kDa

Product No.:	**ELP3010**
Cassette Definition:	**3% Protein 10kDa to 300kDa**
Cassette and Marker:	**3% SDS-Agarose / Marker 03**

Research Use Only

Recommended Sample Load Guidelines

If sample is in high salt buffer (e.g. 7M Urea or 8M guanidine), buffer should be exchanged by dialysis or gel filtration into the provided SDS electrophoresis buffer. Sample and buffer composition will influence sample migration. Results may vary when using alternative buffers. Using a reductant such as TCEP or DTT is recommended.

Recommended Buffer

10-30 mM Tris, pH 7.4 – 8, 0-10 % glycerol, up to 50 mM TCEP

Maximum Load: 350µg in 26µl **Minimum Load:** 100ng in 26µl

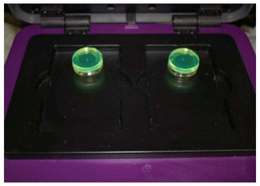
Note: This guide describes the workflow for using an internal marker. Refer to the SageELF operations manual for running timed fractionation.

A. Prepare protein samples

1. Bring the "Loading Solution/Marker-03" mix to room temperature.
2. Bring the protein sample up to 26µl with buffer.
3. Add 4µl of 0.5M TCEP to the protein sample.
4. Heat denature the protein sample at 85ºC for 6 minutes.
5. Combine the 30µl of protein sample with 10µl of "Loading Solution/Marker-03" mix.
6. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.

B. Calibrate the Optics with the Calibration Fixtures

1. Place calibration fixture(s) onto the optical nest(s) as shown.
2. Close the instrument lid.
3. Go to the "Main" tab in the software.



4. Clear the protocol field by pressing the “Clear Run Data” button (if necessary).

5. Press the “Calibrate Detector” button.

6. In the Detector Calibration pop-up window, select the nest(s) to be calibrated.

7. Press “Start”.

8. When calibration is complete, press “Return”.

C. Program a Protocol

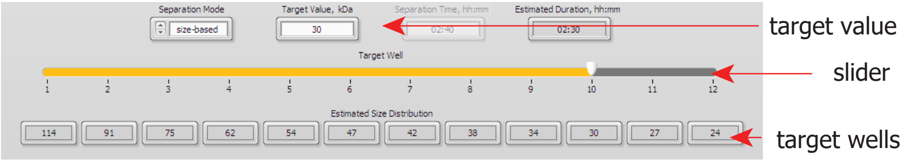
1. Go to the “Protocol Editor” tab in the software.

2. Select “New Protocol” in the menu bar.

3. Select **3% Protein 10kDa to 300kDa** from the “Casssette Definition” drop-down menu.

4. Using “size-based” mode, move the slider to a target elution well number.

5. Enter a value in to the “Target Value” window. This defines the range of fragments that will be collected in the target elution well. The collection range for the remaining wells will be calculated in the software.


Separation Mode: size-based
Target Value, kDa: 30
Separation Time, hh:mm: 02:40
Estimated Duration, hh:mm: 02:30
Target Well: 10

Estimated Size Distribution

1	2	3	4	5	6	7	8	9	10	11	12
114	91	75	62	54	47	42	38	34	30	27	24

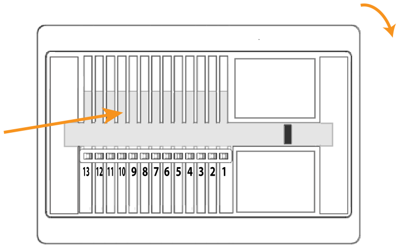
6. The chart below can be used as a guideline to estimate fractionation values.

Est. Run Time (hrs)	Elution Wells 1 (top) to 12 (bottom), Estimated Median Size (kDa)											
	1	2	3	4	5	6	7	8	9	10	11	12
1.1	224	171	105	67	17	18	7	0	0	0	0	0
1.3	291	224	150	109	67	53	38	26	18	11	10	7
1.4	335	298	194	151	117	88	69	52	42	33	27	21
1.5	380	313	239	194	167	123	100	78	67	55	44	35
1.7	410	358	283	236	216	158	131	105	92	77	61	49
1.8	447	410	327	278	266	193	162	131	117	99	78	63
1.9	507	477	372	321	316	228	193	158	142	121	95	77

7. Press “Save As” and name the fractionation protocol.

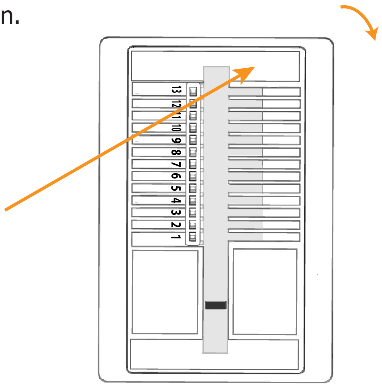
D. Prepare the Cassette(s)

1. **While still taped closed!** Hold the cassette sideways with the elution port side down. Tap to remove air bubbles from beneath the gel fingers.



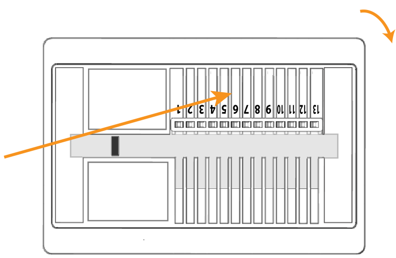
Tap cassette to dislodge bubbles from beneath gel fingers

2. Rotate the cassette in the clock-wise direction. Allow the bubbles to collect in the lower buffer area. Gently tap if necessary.



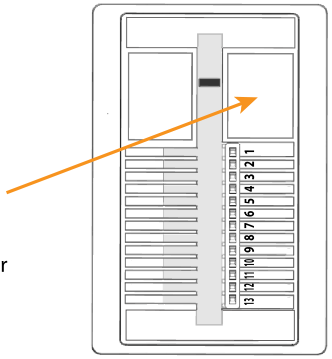
Allow bubbles to aggregate in lower buffer area.

3. Continue to rotate the cassette clockwise, moving as many bubbles as possible to the side buffer area. Tap the cassette firmly to dislodge any bubbles behind elution wells.



Tap to dislodge bubbles from behind elution wells.

4. Continue to rotate the cassette until most of the bubbles have accumulated in the upper buffer area. Place the cassette onto a benchtop.



Continue to move the bubbles until most of the air is accumulated in the upper buffer area.

5. **On a flat bench top:** Peel off the adhesive tape. Grab the tape tab, hold the cassette firmly down, and pull the tape with a steady motion.

6. Remove all buffer from all 13 elution wells (set pipette to 40 µl to completely empty wells). Keep pipette tips vertical in the well to avoid damage to the membranes.

7. Add 30 µl of buffer to all 13 elution wells.